measuring volumes of preferably $\leq 10^{-14}$ 1 by means of fluorescence spectroscopy which device comprises

- a laser beam generation device for the generation of a laser beam with a first wavelength,
- a focusing device for the focusation of said laser beam onto the measuring volume wherein the laser beam is such highly focused within the measuring volume that in essence it exclusively covers the measuring volume,
- a detector device for detecting fluorescence radiation generated due to the laser light excitation of one or more molecules, molecular complexes and/or molecular fragments, and
- a pinhole aperture arranged in the object plane within the beam path of the fluorescence radiation confocally with respect to the measuring volume to limit the quantity of fluorescence radiation to be detected by the detector device wherein said pinhole aperture has a diameter of especially ≤ 100 μm and preferably of ≤ 20 to 30 μm.

86. The device according to claim 85, characterized in that with an image scale of 1 : 100, 1 : 60, or 1 : 40 between the measuring volume and the object plane and with a measuring volume having dimensions of ≤ 0.1 μm in each

direction, said pinhole aperture has a diameter of about 10 μm, 6 μm, or 4 μm, respectively.

87. The device according to claim 85, characterized in that the optics for the laser beam and/or the optics for the fluorescence radiation has a high numerical aperture of preferably $\geq$ 1.2 N.A.

88. The device according to claim 85, characterized in that the measuring volume is distanced from the focusing device by up to 1,000 μm.

89. The device according to claim 85, characterized in that the focusing device has a prefocusing device to prefocus the laser beam and a focusing objective lens to focus the prefocused laser beam onto the measuring volume.

90. The device according to claim 88, characterized in that the distance between the focusing objective lens and the measuring volume is up to 1,000 μm.

91. The device according to claim 89, characterized in that between the prefocusing device and the focusing objective lens a semitransparent mirror is arranged to deflect the prefocused laser beam onto the focusing objective lens.

92. The device according to claim 91, characterized in that the pinhole aperture is arranged at the side of the semitransparent mirror facing away from the focusing objective lens.

93. The device according to claim 85, characterized in that the detector device has at least one and preferably more detectors for detecting the fluorescence radiation.

94. The device according to claim 85, characterized in that between the pinhole aperture and the detector device, there is arranged at least one optical filter and/or at least one imaging lens and/or at least one semitransparent mirror and/or at least one reflecting mirror.

95. The device according to claim 85, characterized by an additional laser beam generation device for the generation of an additional laser beam with a wavelength different from the first wavelength, an additional focusing device for focusing said additional laser beam onto the measuring volume in such high an extent that the additional laser beam in essence exclusively covers the measuring volume, an additional detector device for the detection of fluorescence radiation generated due excitation of one or more molecules, molecular complexes and/or molecular fragments, and a correlator unit which is connected with the two detector devices.

96. The device according to claim 95, characterized by

- a T-shaped support with a first supporting arm (65) and a second supporting arm (74) connected therewith and running perpendicular to the first supporting arm (65),

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- holding devices (83, 84) arranged at the ends at the faces of the second supporting arm (74) for axial guiding and holding of optical elements (lens, filter, mirror, detector) for the two laser beams and the two fluorescence radiations wherein the focused laser beams impinge on a glass slide bearing the measuring volume and being separably arranged preferably halfway between the two ends at the faces of the second supporting arm (74) and held by them,
- wherein the two holding devices (83, 84) can be moved synchronously relative to their respective ends at the faces of the second supporting arm (74) in the direction of the longitudinal extension thereof, the two holding devices (83, 84) are extended in the direction of extension of the first supporting arm (65), and the two laser beams can be deflected by deflecting mirrors and/or semitransparent mirrors (66, 67, 72, 73) through optical openings (69) out of the inside of the first supporting arm (65) onto the optical elements for the laser beams held at the holding devices (83, 84).

97. The device according to claim 96, characterized in that the optical elements for the laser beams are arranged at the inner sides, facing each other, of the two holding devices (83, 84) and the optical elements for the fluorescence

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radiation are arranged at the outer sides, facing away from each other, of the two holding devices (83, 84).

98. The device according to claim 96, characterized in that the one of the focusing objective lenses can be positioned by an adjusting element which is in particular piezoelectrically driven for compensation of an offset of the focuses of said focusing objective lenses.

99. The device according to claim 85, with microscope optics known *per se* for laser focusing for the excitation of fluorescence in a small measuring compartment of a very dilute solution ~~and~~ for confocal imaging of the emitted fluorescence light for subsequent measurement wherein at least one system of optics with high numerical aperture of preferably ≥ 1.2 N.A. is employed, the light quantity is limited by a confocally arranged pinhole aperture in the object plane behind the microscope objective, and/or the measuring compartment is positioned preferably at a distance of up to 100 μm from the observation objective.

100. The device according to claim 99, for the generation of diffraction limiting focusing of a laser beam with a unit generating the measuring signal and an observation unit wherein at the side of generation of the measuring signal an appliance (20) for prefocusing a laser beam (21), a dichroitic mirror (30) for deflecting said laser beam (21), and an additional lens (40) for focusing the laser beam onto the measuring volume are provided and wherein the observation unit

has photon counting appliances (52), a correlation appliance (71), and a multichannel scaler appliance (72), and the measuring signal is optionally processed and/or evaluated in a computer assisted way.

101. The device according to claim 100, wherein the appliances (20) for prefocusing are provided with a lens (22) and an array (23) corresponding to microscope optics wherein a collineated laser beam (21) is focused on the image plane B_1 by a lens L and on the image plane B_2 (first image) by said array (23).

102. The device according to claim 101, wherein said array (23) is provided with an exchangeable arrangement of lenses for the variation of the diameter of the prefocused laser beam (21).

103. The device according to claim 100, wherein a detection unit is constituted by two detectors (53, 54) with a beam splitter (60) partitioning the light (55) emitted from the sample to the detectors (53, 54).

104. The device according to claim 103, wherein the emitted light beam (55) passes imaging lenses (56, 57) and filter elements (58, 59) prior to each of the detectors (53, 54).

105. The device according to claim 100, wherein the detectors (53, 54) detect light of different wavelengths.

106. The device according to claim 100, wherein one or more detector elements are placed in the image plane optionally in the form of a detector array.

107. The device according to claim 100, wherein a pinhole aperture (50) is arranged in the beam path (55).

108. The device according to claim 99, characterized in that two objectives are used which form an angle of > 90° between them.

109. The device according to claim 99, characterized in that continuous lasers having emitted wavelengths of > 200 nm are used as the light source, in particular argon, krypton, helium-neon, helium-cadmium lasers or lasers pulsed with high frequency of ≥ 20 MHZ with a power of ≥ 0.5 mW.

110. The device according to claim 99, characterized in that appliances for single photon counting such as avalanche diode detectors are arranged in the beam path of the emitted light, preferably in the plane of the pinhole aperture, for detecting the emitted light wherein signal analysis is performed by a digital correlator or multichannel counter.

111. The device according to claim 99, characterized in that the measuring compartment is fixed in a sample volume between two capillaries, said capillaries being provided with a chemically inert conductive coating at the outer side, in particular metal vapor deposited coating, especially gold vapor deposited coating on a chromium priming, wherein the conductive coatings are connected with a computer controlled rectified field or an alternating electric field and are conductively connected with each other through the measuring compartment.

112. The device according to claim 99, characterized in that two microscope optics facing each other enclose the measuring compartment.

113. The device according to claim 99, characterized in that an electrophoretic additional device is provided having at least one electrophoresis cell having at least one opening for charging/discharging of the sample to be analyzed and/or a washing solution, a wall electrode, a ring electrode, a Neher capillary, an electrode at the tip of the capillary and a droplet outlet.

114. The device according to claim 113, characterized by an electric trap having a quadrupole element with at least four electrodes, preferably pin electrodes or vapor-deposited electrodes in a wafer configuration wherein preferably a hole of < 1 mm is lined, preferably in combination with at least two additional electrodes in at least a sextupole arrangement wherein the quadrupole element is preferably provided with alternating voltage and a direct voltage is applied to the sextupole electrodes such that the polarity thereof is opposed to the charge of the molecules to be analyzed.

115. The device according to claim 113, characterized in that the sheet for receiving the samples has specific binding properties for molecules due to molecular derivatization, in particular in the form of ion-exchange ligands or affinity ligands, especially oligopeptides, polypeptides, proteins, antibodies or chelating agents, especially iminodiacetic acid or nitrilotriacetic acid ligands, particularly